Mycorrhizae and growth of white fir seedlings in mineral soil with and without organic layers in a California forest

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White fir (*Abies concolor* (Gord. et Glend.) Lindl.) seedlings appear to survive and grow better in mineral soil alone than in mineral soil with organic layers. To determine whether the better growth observed in seedlings in mineral soil (M), compared with those in mineral soil with organic layers (MO), could be related to the incidence of mycorrhizae in their root systems, natural M and MO seedlings were examined from five sites in the north central Sierra Nevada. Statistical analysis of the data showed that seedling dry weight was significantly related to total length of roots, number of main lateral roots, and presence of mycorrhizae on the roots. Dry weight, total length of roots, total number of mycorrhizal tips, and number of mycorrhizal tips per centimetre of roots were significantly higher for M compared with MO seedlings. Age, number of main lateral roots, and number of mycorrhizal tips per centimetre of roots are highly significant in distinguishing M from MO seedlings. The data indicate that mycorrhizae play an important role in the growth of white fir seedlings, and that both growth and presence of mycorrhizae are favored by the absence of organic layers.

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Les plantules de l'*Abies concolor* (Gord. et Glend.) Lindl. semblent survivre et croître mieux dans le sol minéral seul, que dans le sol minéral avec horizons organiques. Afin de déterminer si cette meilleure croissance observée chez les plantules en sol minéral (M), comparativement à celles sur sol minéral avec horizons organiques (MO), pourrait être reliée à l'incidence des mycorrhizes sur leurs systèmes racinaires, les auteurs ont examiné des plantules venues naturellement sur sol M et MO dans cinq stations du centre-nord de la Sierra Nevada.

L'analyse statistique des résultats montre que le poids sec des plantules est significativement relié à la longueur des racines, au nombre de racines latérales principales et à la présence de mycorrhizes sur les racines. Le poids sec, la longueur totale des racines, le nombre total d'apex mycorrhizés et le nombre d'apex mycorrhizés par centimètre de racine sont significativement plus élevés chez les plantules M que chez les MO. L'âge, le nombre de racines latérales principales et le nombre d'apex mycorrhizés par centimètre de racine distinguent significativement les plantules M des MO. Les résultats indiquent que les mycorrhizes jouent un rôle important dans la croissance des plantules de l'*A. concolor* et que la croissance aussi bien que la présence de mycorrhizes sont favorisées par l'absence d'horizons organiques.

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Introduction

Survival of outplanted white fir (*Abies concolor* (Gord et Glend.) Lindl.) seedlings has been poor in California. There may be many reasons for such poor survival, one of which could be inadequate mycorrhizal development. Work done on white fir mycorrhizae (Alvarez and Cobb 1977) has indicated that seedlings from some nurseries do not have mycorrhizae as well developed as those which occur naturally.

It has been observed that white fir seedlings survive best in mineral soil (Gordon 1970; Stack 1965). Our observations indicate that survival and growth of white fir are greater where the organic layers have been removed to expose mineral soil. In India, research on silver fir, *Abies pindrow* Royle, has suggested that poor root and mycorrhizal development is associated with thick humous layers and is one of the factors involved in regeneration failures (Bakshi *et al.* 1972).

The objectives of the research reported here were to test the validity of the observations that white fir seedlings appeared to survive and grow better in mineral soil, and to determine whether the my-

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corrhizal status of seedlings growing in mineral soil, with and without organic layers, was related to the rate of seedling growth. Such information conceivably could be used to improve site preparation and survival of white fir seedlings outplanted in California.

Materials and Methods

Naturally occurring white fir seedlings 1 to 15 years old (indicated by number of stem internodes) were collected in Blodgett Forest, El Dorado County, CA, in the north central Sierra Nevada at about 1400 m elevation. Besides white fir, the overstory contained sugar pine (*Pinus lambertiana* Dougl.), ponderosa pine (*P. ponderosa* Dougl. ex P&C Lawson), Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), incense cedar (*Libocedrus decurrens* Torr.), and black oak (*Quercus kelloggii* Newb.). Seedlings were collected from five different sites: Heliport Road (site 1), Section 5 (site 2), Bacon Creek (site 3), Gaddis Creek (site 4), and Sand Mountain Road (site 5).

Site 1 has a south-facing slope of 3% and is moderately shaded. The organic layers, 3–4 cm thick, were developed from white fir, incense cedar, Douglas fir, ponderosa pine, and oak foliage. Site 2 has an east-northeast slope of 5% and is moderately shaded. The organic layers are similar to those described for site 1. Site 3 faces north-northeast, has a 5–10% slope, and is densely shaded. The organic layers, 3–4 cm thick, were developed from white fir, incense cedar, Douglas fir, and ponderosa pine foliage. Site 4 faces south-southeast, has a 1–2% slope, and is moderately shaded. The organic layers, also 3–4 cm thick, were developed from white fir, incense cedar, Douglas fir, and sugar pine foliage. This is the wettest site and pieces of burned wood were observed in the soil. Site 5 faces west, has a 5–10% slope, and is moderately shaded. The organic layers, 2–3 cm thick, were developed from white fir, incense cedar, ponderosa pine, and oak foliage.

At each site, seedlings growing in mineral soil with organic layers (MO) and in mineral soil without organic layers (M) were collected. Mineral soil with organic layers (MO) represents undisturbed soil in which the L, F, and H layers (Clayton *et al.* 1977) were present. Mineral soil (M) represents disturbed soil in which the O horizons and topmost part of the A_1 horizon has been removed exposing the lower portion of the A_1 horizon.

In the laboratory the roots were gently washed in tap water. Data were recorded for seven seedling varieties: (*i*) seedling age, (*ii*) dry weight of the aboveground portion of the seedling, (*iii*) total length of roots (main and lesser side roots), (*iv*) number of roots (main side roots), (*v*) number of mycorrhizal roots (main side roots with mycorrhizal infections), (*vi*) total number of root tips, and (*vii*) number of mycorrhizal tips. Three additional variables were calculated (*i*) percentage of mycorrhizal roots, (*ii*) percentage of mycorrhizal tips, and (*iii*) number of mycorrhizal tips per centimetre of root length. Several mycorrhizal variables were used in the statistical analyses of the data, although the number of mycorrhizal tips per centimetre of roots was expected to be the most sensitive measurement of prevalence of mycorrhizae in the root system (Slankis 1974) and of the effect of mycorrhizae on the growth of the seedlings.

A total of 165 seedlings was collected, 86 in M and 79 in MO. The data were analyzed first with the full-screen computer program developed at the Pacific Southwest Forest and Range Experiment Station by Nancy X. Norick. The full screen uses multiple regression techniques to find the most significant combination of independent variables for a given dependent variable. The analysis identified the independent variables that were significantly related to the dependent variable, seedling dry weight, which was the best indicator of seedling growth. The actual coefficients relating the dry weight to the other variables were found with a multiple linear regression program. The full-screen program was also used to differentiate among those variables which distinguished M from MO seedlings and those variables which, although different between M and MO seedlings, were merely correlated with the distinguishing variables.

Analysis of variance and Sheffé's test (Kirk 1968) were made on the distinguishing variables and on the seedling dry weight.

Results

The mean ages of the M seedlings on all sites were lower than those of the MO seedlings but all mycorrhizal variables were higher for the M seedlings than for the MO seedlings on all sites except site 4 (Table 1). On site 4, the mean values of the number of mycorrhizal roots and percentage mycorrhizal roots were higher in the M seedlings but number of mycorrhizal tips, percentage mycorrhizal tips, and number of mycorrhizal tips per centimetre of root were lower.

The full-screen analysis indicated that a combination of age, total length of roots, number of main side roots, and number of mycorrhizal tips accounted for 66% of the variability of seedling dry weight. The nondimensional regression coefficients show the proportionate effect of each variable (Table 2). Independent variables shown as not significant in Table 2 were either not correlated with dry weight or were correlated with both dry weight and one or more of the significant independent variables.

Dry weight was examined with a covariance analysis to detect differences in dry weight between M and MO among sites; age was the covariate because seedlings on M and MO were of unequal age (Table 3). The analysis showed that dry weight of M and MO seedlings was significantly different at the 0.05 level of probability. However, dry weights were not significantly different among sites. In four of the five sites and with all seedlings combined by soil condition (M or MO), the means of dry weights adjusted by age were higher for M seedlings than for MO seedlings (Table 4). According to Sheffé's multiple comparison method, the overall dry weight mean difference between M and MO was significant at the 0.05 level but not the differences for individual means of plots.

A second full-screen analysis was made with the soil condition as the dependent variable to determine which seedling variables best distinguished M from MO seedlings. The combination of age, number of

TABLE 1. Numbers and mean values of variables from 165 white fir seedlings collected at Blodgett Forest, CA

Variables	Soil condition[a]	Sites 1	2	3	4	5	Mean of all sites	Minimum	Maximum
No. of seedlings	M	10	10	26	16	24	—	—	—
	MO	12	11	23	13	20	—	—	—
Mean age, years	M	4.6	5.4	5.1	4.8	4.3	4.8	2	14
	MO	7.8	11.0	7.9	5.6	6.4	7.5	1	15
Mean dry weight, g	M	0.72	1.68	1.28	1.04	0.62	1.03	0.09	6.62
	MO	1.13	2.99	1.41	1.44	0.97	1.48	0.07	11.0
Mean length of roots, cm	M	75.6	113.0	69.4	72.3	67.1	75.1	14	254
	MO	74.5	117.0	65.9	71.4	56.3	72.9	9	275
No. of main side roots	M	21.0	23.8	23.4	17.8	19.3	21.4	12	41
	MO	19.4	18.8	16.0	17.0	19.7	17.0	7	35
Mean no. of mycorrhizal roots	M	13.4	14.9	11.9	10.7	10.4	11.8	2	25
	MO	10.9	7.8	5.9	9.5	7.3	7.9	2	18
Mean % mycorrhizal roots	M	65.5	63.3	53.9	60.6	56.2	58.2	6	100
	MO	55.3	44.5	38.3	56.8	48.9	47.5	11	91
Total no. of root tips	M	196	378	161	115	162	182	32	1362
	MO	175	268	192	150	102	171	6	751
Mean no. of mycorrhizal tips	M	110	226	81	61	75	96	2	1086
	MO	98	82	47	68	37	60	2	491
Mean % mycorrhizal tips	M	60.0	51.2	51.3	47.8	48.5	50.9	3	91
	MO	52.5	30.9	29.5	50.3	38.7	38.9	2	93
Mean no. of mycorrhizal tips per centimetre of roots	M	1.73	1.58	1.15	0.81	1.26	1.23	0.042	4.28
	MO	1.19	0.61	0.68	0.91	0.76	0.81	0.040	4.18

[a]M, mineral soil; MO, mineral soil with organic layers.

main side roots, and number of mycorrhizal tips per centimetre of roots was highly significant in distinguishing M from MO seedlings with each of these variables individually significant at the 0.005 level. Two other variables, total length of roots and total number of mycorrhizal tips, were significantly higher for M seedlings (Table 3) but became nonsignificant when included in the equation because they were correlated with one or more of the three distinguishing variables.

Analysis of covariance by soil condition and site, with age as the covariate, indicated that number of main side roots was significantly different at the 0.001 level for M seedlings compared with MO seedlings but there were no significant differences among sites (Table 3). The age-adjusted mean of all M seedlings

TABLE 2. Significance and nondimensional regression coefficients of variables associated with dry weights of 165 white fir seedlings from Blodgett Forest, CA

	Soil condition	Site	Age, years	Total length of roots, cm	No. of main side roots	No. of mycorrhizal roots	% mycorrhizal roots	Total no. of root tips	Total no. of mycorrhizal tips	% mycorrhizal tips	No. of mycorrhizal tips per centimetre of roots
Significance of dependent variables[a]	NS	NS	0.0005	0.0005	0.002	NS	NS	NS	0.004	NS	NS
Nondimensional regression coefficients[b]	—	—	0.769	0.987	−0.671	—	—	—	0.158	—	—

[a]NS indicates $P > 0.05$.
[b]Coefficients C_i of equation $W/\overline{W} = -0.243 + C_1 X_1/\overline{X}_1 + C_2 X_2/\overline{X}_2 + \ldots C_i X_i/\overline{X}_i$.

TABLE 3. White fir seedling variables tested by covariance analysis for differences between soil conditions and among sites for seedlings collected at Blodgett Forest, CA

	Probabilities of significant differences[a]		
	Soil condition (M or MO)	Site	Age
Dry weight, g	0.95	NS[b]	0.999
Total length of roots, cm	0.999	0.95	0.999
No. of main side roots	0.999	NS	0.999
Total no. of mycorrhizal tips	0.999	0.95	0.999
No. of mycorrhizal tips per centimetre of roots	0.999	0.95	NS

[a]All interactions were not significant.
[b]NS indicates $P < 0.95$.

combined was significantly higher at the 0.001 level compared with that of all MO seedlings (Table 4).

The numbers of mycorrhizal tips per centimetre of roots were compared by soil condition and site with a two-way analysis of variance (Table 3). The difference between M and MO seedlings was significant at the 0.001 level, whereas the differences among sites were significant at the 0.05 level. The individual and combined means of number of mycorrhizal tips per centimetre of roots (Table 4) were compared using Sheffé's method. The combined mean of M seedlings for sites 1, 2, 3, and 5 was found to be significantly higher at the 0.05 level compared with the combined mean of the MO seedlings for the same sites. The M and MO seedlings were not significantly different in site 4. The difference among sites indicated by the analysis of variance for mycorrhizal tips per centimetre of roots was primarily due to a value for M seedlings on site 4 that was significantly lower at the 0.05 level than the highest value for M seedlings on site 1.

Discussion

It has been suggested (Meyer 1973, 1974; Harvey *et al.* 1976) that the conditions for mycorrhiza formation are better in the humous than in the lower mineral soil. This statement is based mostly on research done with old trees, the root systems of which extended into the different soil horizons. The results of our comparative study of white fir seedlings growing in mineral soil (M) and in mineral soil with organic layers (MO) indicate that seedlings grow better in the mineral soil.

Dry weight, total length of roots, total number of mycorrhizal tips, and the number of mycorrhizal tips per centimetre of roots were higher for M than MO seedlings. Soil conditions affect the dry weight of the seedlings by affecting variables such as total length of roots and total number of mycorrhizal tips which have a direct effect on seedling dry weight. Total length of roots has a stronger effect on seedling dry weight than total number of mycorrhizal tips.

A combination of three variables (age, number of main side roots, and number of mycorrhizal tips per centimetre of roots) is highly significant in distinguishing M from MO seedlings. Other variables found to be different between M and MO seedlings were explained by correlations with one or more of these variables.

Bakshi *et al.* (1972), also working with fir seedlings, found that silver fir seedlings develop better root systems in mineral soil than in humous and mycorrhizae developed in both layers once the roots reached the mineral soil beneath. These researchers interpreted their findings to mean that nutrients were

TABLE 4. Means of age-adjusted dry weights and of variables distinguishing M from MO white fir seedlings, from five sites at Blodgett Forest, CA

Variables	Soil condition	Site 1	Site 2	Site 3	Site 4	Site 5	Mean, all seedlings combined
Age, years	M	4.6	5.4	5.1	4.8	4.3	4.8
	MO	7.8	11.0	7.9	5.6	6.4	7.5
Age-adjusted dry weight, g	M	1.22	1.91	1.62	1.47	1.23	1.49[a]
	MO	0.55	1.41	0.82	1.59	0.88	1.00[a]
Age-adjusted total no. of roots	M	21.4	24.0	23.6	18.1	19.8	21.3[b]
	MO	19.0	17.6	15.5	17.1	15.6	16.6[b]
No. of mycorrhizal tips per centimetre roots	M	1.73[a]	1.58	1.15	0.81[a]	1.26	1.43[a,c]
	MO	1.19	0.61	0.68	0.91	0.76	0.81[a,c]

[a]Values differ at $P = 0.05$.
[b]Values differ at $P = 0.001$.
[c]Mean of site means for sites 1, 2, 3, and 5.

available in the mineral soil. Nutrient availability does not appear to be the only factor involved. We think a difference in the mycorrhizal fungi is involved. In the M seedlings the predominant types were brown, whereas in the MO seedlings, although the same brown types were present, there was also a diversity of other types (Alvarez and Cobb 1977). It is known that some mycorrhizal fungi affect the growth of the host more favorably than others (Marx 1975; Mikola 1973; Trappe 1977). Hence, it is possible that the mycorrhizal fungi predominant in the M seedlings are more beneficial to the growth of white fir seedlings than those present in the MO seedlings. Perhaps the mycorrhizal fungi predominant in the M seedlings are poor competitors, sensitive to harmful substances found in the organic layers (Meyer 1966). These toxic substances could in themselves alter the physiology of the MO seedlings making them less vigorous and susceptible to the other mycorrhizal fungi.

We observed that in both M and MO seedlings most mycorrhizae occurred in the upper third of the root system (about 10 cm) and the incidence of mycorrhizae decreased with the increasing depth, an observation that supports other research (Dominik 1966). In future studies a more accurate measurement of the effect that mycorrhizae have on seedling growth could be obtained if the root system were to be divided in portions by soil depth and the number of mycorrhizal tips per centimetre of roots was computed for each one of these portions.

Additional factors such as nutrient availability, soil temperature, and soil microflora need study. Further research is also needed in the types of mycorrhizae and environmental factors that affect the incidence of mycorrhizae in seedlings growing in mineral soils with and without organic layers. This area of research can have important practical applications in decisions involving site preparation before outplanting.

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